Principles Underlying the Serum Diagnosis of Typhoid Fever and the Methods of its Application.

Presented in Opening the Discussion on Serum Diagnosis in the Section on Practice of Medicine, at the Forty-eighth Annual Meeting of the American Medical Association, at Philadelphia, Pa., June 1-4, 1897.

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I comply the more readily with the suggestion of the Chairman of this Section that my remarks in opening this discussion on the "Serum Diagnosis of Typhoid Fever," shall relate to the general principles of the method, inasmuch as the results obtained by this method at the Johns Hopkins Hospital will be presented in the course of this discussion by Dr. Block, and others will relate the results of their personal experience.

Before the introduction of the Widal method of diagnosis the discovery and subsequent studies of the typhoid bacillus had been comparatively barren of results available to the general practitioner of medicine. In this respect the typhoid bacillus afforded a marked contrast to many other pathogenic microörganisms, notably the tubercle bacillus, the diphtheria bacillus and the malarial parasite.

For various reasons improved methods of diagnosis of typhoid fever are most welcome to the practitioner. Inasmuch as the prevalence of this disease is a just reproach to the sanitary conditions of a locality, there has often been a readily explicable, if not creditable, reluctance on the part of some physicians, especially in public institutions, country towns and summer resorts, to make the diagnosis of typhoid fever even in clear cases. Moreover, notwithstanding all that has been written on the subject, knowledge of the frequent deviations of typhoid fever from the classical type, amounting sometimes to entire absence of all features of this type, can hardly be said to have

become sufficiently common property of the medical profession in this country. The differential diagnosis of typhoid fever from certain other diseases, such as acute miliary tuberculosis, tuberculous peritonitis and meningitis, acute ulcerative endocarditis and various other septic affections, may, for a time at least, be most difficult or impossible, even to skilled diagnosticians. In children the disease is prone to anomalous manifestations. Since the discovery of the malarial parasite there is no longer any excuse for confounding typhoid and malarial fevers. A method of positive diagnosis of typhoid fever will elucidate the much disputed nature of many short and mild febrile diseases and of certain fevers of warm climates, as in the southern part of this country.

Before the introduction of serum diagnosis, numerous attempts had been made to utilize the presence of the typhoid bacillus for purpose of diagnosis. One can obtain with considerable regularity cultures of the typhoid bacillus by hypodermic puncture of the spleen in typhoid patients; but this procedure is not wholly without danger. Anyone who has seen at autopsy a swollen, soft typhoid spleen with its capsule distended to the utmost and ready to burst the moment it is lifted from the body, would certainly hesitate to insert even a hypodermic needle into such a spleen during life. Instances are on record in which such puncture of the spleen has given rise to severe intraperitoneal hemorrhage. Cultures from roseola spots, suggested by Neuhauss in 1886, yield uncertain results. Cultures from the blood give positive results in some cases, especially if large amounts be used. Cultures from typhoid stools reveal the presence of the specific bacillus in many cases if the examination be made with sufficient patience and care. The introduction of the Elsner and of the Capaldi nutritive media marks a distinct advance in this method. None of these procedures, however, in their present form afford simple and ready methods of diagnosis in the routine of hospital and private practice.

History.—The Widal method of diagnosis is based on the application of scientific discoveries made before Widal's first publication, June 26, 1896. As a controversy for priority, attended with no little bitterness, has arisen, it may be well to state the main historic facts. Like so many other bacteriological discoveries of practical utility, this one is the outcome of investigations concerning immunity.

In 1889, Charrin and Roger noticed that the bacillus pyocyaneus grows in the form of clumps in the undiluted serum of animals rendered immune from this bacillus, whereas in normal serum it grows with diffuse clouding of the medium. This is the first observation of the property of immune serum to cause agglomeration of specific bacteria.

In 1891, Metchnikoff observed the same phenomenon together with immobilization of the bacteria in cultures of the vibrio Metchnikovi, and also clumping of the pneumococcus, in their immune sera and he said: "This fact, presenting a general importance, should be investigated more fully." He did not, however, pursue the investigation, and as he failed to find the same behavior of the hog cholera bacillus[1] in its immune serum, he seems to have abandoned the idea first expressed as to the general importance of the phenomenon.

In 1893, Issaeff, in the Pasteur Institute, and in 1895, Washbourn, confirmed Metchnikoff's observation as to the pneumococcus, and in 1894 Issaeff and Ivanoff, in Koch's Institute, made the same observation regarding the vibrio of Ivanoff.

In 1894, Pfeiffer, in conjunction with Issaeff, published his important studies on immunity from Asiatic cholera, in which he showed that cholera spirilla introduced into the peritoneal cavity of immunized guinea pigs, or introduced together with immune serum into the peritoneal cavity of normal guinea pigs, quickly lose their motility and break up into small granules. This behavior in the animal body, known as the "Pfeiffer phenomenon," and demonstrated in 1896 by Pfeiffer and Kolle also for typhoid infection, became the subject of investigation by others, which led to the recognition of the importance and general bearings of the agglutinative reaction of specific sera. The Pfeiffer phenomenon is distinct from the agglutinative reaction upon which the method of serum diagnosis to be here considered is based, and Pfeiffer himself can not be credited with a clear recognition of the diagnostic importance of the latter reaction before it was made manifest by

[1] As a matter of fact, the hog cholera bacillus is agglutinated and immobilized by immune hog-cholera serum, as has been shown by Dawson (New York Med. Journal, Feb. 20, 1897). This is an additional proof, if any were needed, of the fact repeatedly insisted upon by the writer and others in this country, that Metchnikoff's so-called hog-cholera bacillus is not the genuine hog-cholera bacillus discovered and described by Theobald Smith (Welch and Clement, Proceedings of the 30th Annual Convention of the U. S. Veter. Med. Assoc. and 1st Veterinary Congress of America, October, 1893).

the work of others. He, however, directed attention to the diagnostic employment of serum with his reaction, and his discoveries formed the basis for the later work on the agglutinative reaction and, therefore, they occupy an important position in the history of serum diagnosis.

In 1895. Bordet, in studying the conditions of production of the Pfeiffer phenomenon outside of the animal body, noted that if a small quantity of immune serum be added to a suspension in salt solution or bouillon of cholera spirilla these lose their motility and become agglomerated. The significance of Bordet's observation is that he was the first to dilu e the serum. His interest, however, was chiefly in the determination of the conditions causing the disintegration of the spirilla by cholera serum, and it can not be said that, at this time, Bordet had a clear perception of the importance and general significance of the agglutinative reaction.

January 3, 1896, Durham presented to the Royal Society a paper giving the results of investigations in Gruber's laboratory in Vienna. This communication embodies the first thorough and systematic study of the agglutinative and immobilizing properties of immune serum outside of the animal body. In this and the rapidly following papers of Gruber and Durham the real importance and general characters of this reaction with immune serum were for the first time made clear. The macroscopic and the microscopic tests, the importance of dilutions, quantitative estimations of agglutinative power, the value of the test for the differentiation of bacterial species and for the determination of a previous attack of cholera or typhoid fever, and many other details were described.

It seems but a small step to determine whether a reaction which had been demonstrated to characterize the serum of animals and human beings which have recovered from an infection may not also be present during the period of infection, but upon this step depended the applicability of the reaction as a method of clinical diagnosis. It was Widal who took it and thereby made available for the diagnosis of an infection a reaction which had previously been thoroughly worked out by Gruber and his collaborators so far as immune serum is concerned.[2] Widal's first communication was presented to the Société Médicale des Hôpitaux on June 26, 1896. This first paper has been followed by numerous important contributions by Widal and others to the same subject.[3]

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[2] It appears that Grünebaum, working in Gruber's laboratory, had before Widal's first publication determined the agglutinative property of the blood serum during the period of typhoid infection, but the results of his investigations were not published until after several papers by Widal and others had appeared.

[3] A summary of their own work on serum diagnosis, as well as that of others, with references to literature, is presented in a recent elaborate paper by Widal and Sicard in the Annales de l'Institut Pasteur, 1897, No. 5.

Nature of the agglutinative property and reaction.—As the result of infection with many bacteria or of intoxication with their products, the blood, even when highly diluted, acquires the property of causing loss of motility and clumping together of the specific bacteria concerned in the infection or intoxication. The clumping is called by Gruber agglutination, and is attributed by him to the presence of substances to which he has given the name agglutinins. He supposes that these agglutinins make the gelatinous capsules of the bacteria swell up and thereby stick the bacteria together. Although there is no proof of this theory, the names "agglutination," to designate the phenomenon, and "agglutinins," for the supposed substances causing it, have been widely adopted.

In the case of motile living bacteria two phenomena characterize the complete reaction, paralysis or immobilization of the bacteria and clumping. Usually these two phenomena go hand in hand, but sometimes there is loss of motility with little clumping or clumping without much cessation of motion, so that the opinion has been expressed that the paralyzing and the agglutinative substances are not identical. The more common deviation from the usual course of the reaction is the occurrence of clumping, with partial preservation of motility.

The agglutinative serum reaction appears to be of wide, although not universal, application, both for motile and non-motile pathogenic bacteria, having been demonstrated for typhoid, Asiatic cholera, pneumococcus infection, tetanus, pyocyaneus disease, glanders, hog-cholera, Malta fever, colon infection, proteus infection, psittacosis, and several other infections.

The change in the blood upon which the reaction depends is doubtless a specific one in the same sense as are the antitoxic, lysogenic, and other specific alterations caused by the action of definite bacteria or their products. It is upon this specificity that the diagnostic value of the reaction is based. It is true that, as in the case of the antitoxic, lysogenic and other

protective modifications of the fluids of the body, the normal blood may possess in some degree the same property, so that the specific character of the change may not be apparent without resorting to considerable dilution of the blood or serum. Normal blood may agglutinate, to some degree, not only the typhoid bacillus but various other bacteria. The specificity of this change resulting from infection with a given microörganism is made apparent by increase of the agglutinative power of the serum only for that microörganism, or to some extent also for closely allied microörganisms. The increase of reaction in some degree with closely allied bacteria, does not militate against the specificity of the change, for it is only an expression of the natural affinities between varieties and races of organisms, as between the typhoid bacillus and the bacillus of psittacosis, or between the cholera spirillum and certain other spirilla, or between the varieties of proteus bacilli or of colon bacilli. The development of the specific agglutinative properties of the blood in typhoid fever affords additional proof, if any were needed, that the bacillus typhosus is the specific cause of this disease.

The blood acquires the specific agglutinative power at a variable period, usually within a few days, after the entrance of the pathogenic microörganism or its products. This power tends to increase, but with much irregularity, during the course of the infection, and gradually to diminish and finally to disappear weeks, months, or it may be years after recovery from the infection. Widal lays much emphasis on the reaction being one of infection and not of immunity. Still it is to be noted that by following procedures for raising experimental immunity to great heights, there may be a corresponding rise of agglutinative power, whereby degrees of this power may be attained which are entirely unknown during natural infections. Thus, Widal, by successive inoculations of an ass, has secured typhoid serum with an agglutinative strength of 1 to 43,000, and Salimbeni has obtained, experimentally, cholera serum

agglutinating in a dilution of 1 to 50,000. Gruber speaks even of immune sera which agglutinated distinctly in a dilution of 1 to 500,000. There is, however, no necessary correspondence between the height of immunity and that of agglutination; especially may the latter lessen or disappear when the former is preserved. In the light of the experimental results, and for other reasons, it seems to me somewhat misleading to designate the reaction as merely one of the period of infection.

We are not informed as to the nature of the relationship between the agglutinative and the protective properties of the blood. Gruber has based a new theory of immunity on the agglutinative reaction, but this theory is opposed by many facts and can not be accepted. The agglutinative property has been shown to be distinct from the bactericidal, lysogenic, antitoxic and other known protective properties of the blood. At present we have no satisfactory evidence that the agglutinative reaction is concerned in any of the defensive mechanisms of the body. According to Salimbeni, whose results, however, are not in entire accord with those of Durham, agglutination of bacteria does not take place within the animal body, although it has been demonstrated that the living blood plasma possesses the agglutinative property.

We do not know the origin or nature of the so-called specific agglutinating substance, save that in some way it results from the activities of bacteria or their products within the living body. Gruber believes that it is derived from the bodies of the bacteria through the agency of the cells. Bordet considers that it is secreted by leucocytes. Experiments of Widal and Sicard, and of Achard and Bensaude, indicate that it is not secreted by leucocytes outside of the living body, but there is nothing which shows that it may not be formed by cells within the body.

The agglutinative substance is generally believed to be a proteid, as it is precipitated from blood plasma with fibrinogen and globulin, and from milk with

lacto-globulin and casin, but it is possible that it is simply mechanically retained by these albuminous precipitates. In typhoid fever it may be absent from albuminous urine and may be present in urine which gives no reaction for albumin (Widal). It behaves like an albuminous substance as regards filtration and dialysis.

The question whether it is strictly proper to speak of agglutinative, bacteriolytic, antitoxic, substances in the blood is a legitimate one. They have never been isolated as chemical substances. Behring has expressed the opinion that isolation of antitoxin will never be accomplished, for, in his opinion, it is a force pertaining to highly organized material, and there is no more possibility of separating it as a substance than of isolating the magnetic force from an iron magnet. In all probability these various properties, agglutinative, bacteriolytic, antitoxic, belong to the same general category, and we may look upon the agglutinative property also as a physical one which proteid substance may acquire as the result of the activities of bacteria or their products, and no more separable in the form of a chemical substance than is electricity.

The agglutinative property is quite resistant to injurious agencies. It survives desiccation of the blood or serum for months, and may persist for months in blood serum, even when this is seriously contaminated with microörganisms. It is not destroyed by sunlight, unless overheated. It is weakened by prolonged heating at 60 degrees C., and is annulled by heating for ten minutes at 75 to 80 degrees C.

The agglutinative property in typhoid fever is present in maximum amount in the blood, being somewhat greater in the blood plasma than in the blood serum. It is found in blister serum in essentially the same strength as in blood serum. In other fluids, as the pleural, peritoneal, pericardial, inflammatory and œdematous, it is in smaller and variable amount. In milk and colostrum it is present in marked degree. It is weak and inconstant in the urine, bile and aqueous humor.

It has been found in tears naturally secreted, but is said to be absent from those provoked by irritants. It may be present in typhoid stools (Block). It has not been found in the cerebrospinal fluid or the fluid in the seminal vesicles. It has not been positively determined whether the distribution of the agglutinative property in the various humors of the body outside of the blood, can be explained wholly by processes of filtration and diffusion from the blood plasma, although these are doubtless the main factors.

The agglutinative reaction may or may not be obtained with the blood of a fetus or new-born infant of a mother with typhoid fever. Chambrelent and Saint-Philippe consider that the presence or absence of the reaction in the fetus depends on whether or not the typhoid bacilli break through the placental barrier from mother to fetus and cause infection of the latter. Further investigations are needed to determine this point and thus to decide whether the agglutinative property is passively or actively acquired by the fetus. For it has been shown that this property, like immunity, not only may be actively acquired as the result of infection or intoxication, but may be passively transmitted by injection of agglutinative serum, the reaction in the latter case appearing promptly without symptoms of infection. being relatively slight, and disappearing after a short time.

Courmont thinks that the development of typhoid bacilli in a fluid robs it of agglutinative power. Thus he has found that the vegetation of the bacilli in typhoid serum deprives it, in a few days, of the agglutinative property, and that blood obtained, post-mortem, from the spleen, liver and mesenteric glands, organs in which the typhoid bacilli are especially abundant, is poorer in agglutinative power than that from other parts. Ménétrier found in a case of typhoid fever that the pleural exudate, which usually gives the agglutinative reaction, did not do so, and that it contained typhoid bacilli in large number. It has been suggested that these observations may shed light on

the exceptional cases of typhoid fever with absence of the specific serum reaction. Flexner has shown that, occasionally, the typhoid bacilli develop in the blood in such large numbers as to produce a genuine typhoid septicemia. Still these observations, interesting as they are and deserving further investigation, must be interpreted with caution, for Widal has found a purulent exudate from an immunized ass, swarming with typhoid bacilli, to present, even after fifteen months' preservation, an agglutinative power of 1 to 13,000, the power of the blood serum of the same animal kept for the same length of time being 1 to 14,000.

We have no satisfactory explanation of the production of the phenomenon of agglutination by specific serum. Gruber's explanation already mentioned has received no confirmation. The phenomenon occurs with non-motile as well as with motile bacteria, with dead as well as with living organisms. Typhoid bacilli killed by formol in weak solution, or by heating for five minutes at a temperature of 56 degrees C., are about as sensitive to the reaction as are living bacteria, and retain their agglutinability for a long period (Widal). The phenomenon, therefore, is a physical rather than a vital one, although probably dependent in some way on the protoplasmic constitution of the bacterial cell. Salimbeni, as has been stated, found that the phenomenon does not occur in the living body of immunized animals. He also found that the presence of atmospheric air greatly favors the reaction, it requiring much more concentrated serum and a longer time to bring about the phenomenon in a vacuum than when the fluid is exposed to the air. Widal has shown that other physical conditions, particularly contact with objects, such as the surface of slide or cover-glass, and partial evaporation, favor the production of the reaction.

Agglutinative serum is often likewise bactericidal and bacteriolytic, but the agglutinative reaction is independent of the bactericidal and is manifest with dilutions of the serum which annul the bactericidal

power. In such dilutions the agglutinated and immobilized bacteria are not altered morphologically, or in staining properties, or in pathogenic power, or in any other way, so far as has been determined. Whether the temporary inhibition of bacterial growth in agglutinative serum is dependent on the agglutinative or on some other property of the serum is not known.

Methods of making the serum test for typhoid fever.—Widal, in his first communication, described both the slow or macroscopic and the quick or microscopic methods. For each of these he recommended a dilution of one part of blood or blood serum to ten parts of the fluid containing the culture. The macroscopic method consists in adding the blood or serum to be tested either to a young bouillon culture of the typhoid bacillus or to sterile bouillon which is then at once inoculated with the bacillus. In the former case the reaction with typhoid serum appears usually within two or three hours and consists in clarification of the previously turbid fluid, and the formation of a clumpy sediment composed of accumulated bacilli. In the latter case the tube is placed in the incubator and within fifteen hours the reaction is manifested by growth of the bacilli in the form of a sediment at the bottom of the tube, the fluid remaining nearly or quite clear.

The microscopic test, to which Widal gave the preference, is made by mixing the blood or serum with a young bouillon culture or with a suspension in bouillon or salt solution of a fresh growth of the typhoid bacillus and examining a drop or two of the mixture at once under the microscope. With a dilution of 1 to 10 this microscopic typhoid reaction appears, as a rule, immediately or within a few minutes, and is evidenced by loss of motility and by clumping of the bacilli into masses of various sizes and shapes.

Widal obtained the blood either with a sterilized hypodermic syringe from a vein of the arm, or by pricking the finger. It may also be conveniently

obtained by pricking the lobule of the ear. A few drops of blood suffice for collecting the necessary amount of serum; indeed a single drop will do for the reaction. The blood may be collected in a small test tube where, usually in a few minutes, it clots. The separation of the serum may be facilitated by passing a sterilized platinum needle between the glass and the clot, or by the centrifuge, or the blood may be collected and allowed to clot in a slanted tube, which is then placed upright, the separated serum trickling to the bottom.

In a communication made July 31, 1896, Widal said that results equaling those with blood serum can be obtained with blister serum, and this procedure has been employed with much satisfaction by the Health Department of New York city.

At the same time Widal called attention to the preservation of the agglutinative property in dried blood and serum. Wyatt Johnston deserves the credit of developing the test with dried blood and for introducing the method of serum diagnosis into the work of municipal laboratories. The dried-blood method, which has been used far more extensively in Canada and this country than in Europe, possesses certain manifest advantages, especially ease of collection, freedom from subsequent contamination and readiness of transportation, and it has given excellent results in the hands of Johnston and others. The principal objection, and this is of considerable importance when precise results are desired, is the difficulty of obtaining accurate quantitative dilutions with the use of dried blood.

Several observers, including Breuer, Haedke, Du Mesnil de Rochemont, Scheffer, have expressed the opinion that the macroscopic method is more trustworthy than the microscopic. This I believe to be an error and to be due to unfamiliarity with all of the conditions essential for the accurate employment of the microscopic test. The latter is more delicate, prompt and precise than the macroscopic reaction, and

requires less care in respect to accidental contamination.

A year's experience with the method of serum diagnosis of typhoid fever has led to a general consensus of opinion as to its great value. It has, however, been recognized that certain precautions in the application of the test are necessary in order to avoid mistakes. Numerous modifications of the original methods have been suggested, the most important relating to quantitative determinations. In considering the value and practical utility of such modifications of the test, several points should be borne in mind. Practically all of the methods recommended by competent investigators have given good results in the great majority of cases. For clinical purposes it is desirable that neither the method of obtaining and collecting the blood nor that of conducting the test should be made more difficult and complicated than is absolutely necessary. Methods which may be essential for exact scientific work, where every possible source of fallacy is to be avoided, may not be the best for the routine examinations of a clinical or a municipal laboratory. Where absolute accuracy is not obtainable it is upon the whole better that the method should err on the side of now and then including a non-typhoid case than in excluding cases of genuine typhoid fever. With due allowance for such considerations as these, we must welcome all efforts to give greater precision to the methods of serum diagnosis and to determine the capabilities of these methods and their possible sources of error. In exact quantitative work with the serum test the most important points to be considered are the characters of the culture, the dilution of the serum, the time limits, the criteria of the reaction and certain physical conditions influencing the reaction.

Characters of the culture.—There has been considerable difference of opinion as to whether cultures of the typhoid bacillus obtained from different sources are equally sensitive to the agglutinative reaction. Widal, Durham, Stern and C. Fraenkel, who have all

had large experience with different cultures, have found only unimportant and inconstant differences in susceptibility to the reaction. The fact determined by Pfeiffer that the less virulent the culture, the greater the sensitiveness to the lysogenic reaction (Pfeiffer's phenomenon), seems to have been considered by many without sufficient investigation to be equally applicable to the agglutinative reaction. Kolle, without however presenting sufficient evidence, emphasizes the greater susceptibility of cultures with weakened virulence to agglutination. The most satisfactory evidence on this point is furnished by Kühnau, who made a careful comparative study of the behavior with the serum test of a non-virulent and a virulent typhoid culture, and found the former to react much more intensely with normal and typhoid sera. He, therefore, lays stress on consideration of the virulence of the culture in quantitative work with the serum test. In view of the conflict of opinion further investigations upon this question are needed.

It cannot be doubted that several observers have had to do with typhoid cultures which presented distinct differences in susceptibility to the agglutinative reaction. Especially worthy of consideration, although not wholly in accordance with some results of others, are the observations of Johnston and McTaggart, confirmed by Appel and Thornbury, that solutions of dried blood are more potent than serum in agglutinative power, although not in paralytic effect, and that such solutions from non-typhoid cases are prone to give partial (pseudo-) reactions with frequently transplanted typhoid cultures, whereas this difficulty is largely overcome by using fresh cultures planted from stock cultures a month old. Hence they strongly recommend for the dried-blood method cultures of the latter character. They, as well as other writers, likewise emphasize the importance of considering the composition of the culture medium, which should be favorable to vigorous growth and not too strongly alkaline.

Only young cultures should be used, preferably not over twelve to eighteen hours old, if grown in the incubator. Older room cultures can be used. Old cultures agglutinate more readily than young ones. Either bouillon cultures or suspensions in bouillon from solid cultures may be employed. There is no difficulty in securing uniform suspensions of isolated, actively motile typhoid bacilli, especially from young cultures on dried out agar. In every case it is of prime importance to make a control examination of a drop from the same part of the culture or suspension which is used for the test and at the time of making the test in order to be sure that there are no preëxisting clumps, that the bacilli are actively motile, and that the culture is not contaminated.

Stern suggested that the concentration of the suspension, that is the number of bacilli in it, may be a factor meriting consideration, and Kühnau and Block have shown that this is the case. Weak suspensions are more readily agglutinated and paralyzed than stronger ones. Hence, Kühnau recommends the use of suspensions of known concentration, which can be approximately secured without much difficulty. He uses a suspension in bouillon of a fifteen-hour virulent agar culture (grown in the incubator) containing about one hundred and twenty million bacteria in a cubic centimeter.

Dilution of the serum.—Inasmuch as normal and non-typhoid blood may possess distinct agglutinative property, especial importance is attached to dilution of the serum, in order to avoid mistaking the normal reaction for one of typhoid fever. The opinion has been widely expressed that the dilution recommended by Widal, 1 to 10, is too low, and that a dilution should be used which is not known ever to give a reaction with non-typhoid blood. The fixation of the upper limit of such a dilution has been placed gradually higher and higher, thus by du Mesnil at 1 to 25, by Kolle 1 to 30, by Grünbaum 1 to 33, by Stern 1 to 40, by Kühnau 1 to 50. Even if it should

be admitted that a reaction in non-typhoid cases with these higher dilutions is ever of such a character as might mislead an experienced observer, its occurrence is, according to most observers, very exceptional.

The question arises whether the adoption of a dilution of say 1 to 50 as the standard, would result in the exclusion of genuine typhoid cases from the diagnosis. Widal divides the typhoid cases in which he has measured the agglutinative power of the blood into five groups: *a*, those with very weak power, less than 1 to 100 (four cases); *b*, with weak power, between 1 to 100 and 1 to 200 (nine cases); *c*, with medium or average power, from 1 to 200 to 1 to 500 (eight cases); *d*, with high power, from 1 to 500 to 1 to 2,000 (nine cases); and *e*, with very intense power, exceeding 1 to 5,000 (three cases). In only one case did the agglutinative power not rise over 1 to 40, it being 1 to 30 on the twentieth, and 1 to 40 on the twenty-second day of the disease. In one case, Widal found the strength to be 1 to 12,000. In nineteen cases measured by Stern, the agglutinating strength was never less than 1 to 50. C. Fraenkel found the average to lie between 1 to 100 and 1 to 200, sometimes reaching 1 to 5,000. Out of seven cases Kühnau found two in which the serum was active only in dilutions less than 1 to 50, it being 1 to 30 in one, and 1 to 20 in the other case, but in both he made a positive diagnosis of typhoid fever in consequence of disparity of the action of the serum on the colon bacillus and the typhoid bacillus. From the observations thus far reported, although they are insufficient in number for definite conclusions, there would seem to be only small liability of failure to recognize genuine typhoid cases by resorting to dilutions of 1 to 40 or 1 to 50, but unquestionably a few cases would escape recognition, and for this reason lower dilutions should also be used, and if those between 1 to 10 and 1 to 50 give decided reaction there should be, at least, suspicion of typhoid fever.

It is not, therefore, to be recommended that one

should make the test with only high dilution, such as 1 to 50. The negative result of a preliminary test with equal parts serum and culture suffices to exclude typhoid reaction. The examination, if positive, may then be made with a low dilution of the serum and for this Widal's recommendation of 1 to 10 or 1 to 15 may be well adopted. If with this dilution the microscopic reaction is complete and almost immediate, as is often the case, there is practically no risk in making a positive diagnosis. But for absolute certainty and above all in cases where the result of the reaction is not prompt, complete and unmistakable, higher dilutions should be employed; if the amount of serum permits only one such, it may be 1 to 50, but preferably intermediate dilutions should also be made, and it is desirable, if not absolutely necessary, to try dilutions higher than 1 to 50. For making the dilutions there are various simple technical procedures, which involve but little expenditure of time and labor and only small quantities of serum, as, for example, that recommended by C. Fraenkel. An accurate fixation of the upper limit of agglutinative power is often tedious and not generally necessary in diagnostic work. A positive diagnosis of typhoid fever, based exclusively on the test with a low dilution, in a case which subsequently proves not to be typhoid should not be considered as in any way invalidating the results of an accurate employment of the method of serum diagnosis.

It is self-evident that the employment of varying degrees of dilution of the serum, without at the same time taking into consideration other factors which influence the reaction, has little sense and does not constitute, in itself alone, an accurate method of mensuration of agglutinative power.

Time limits.—As the rapidity with which the reaction appears and progresses generally varies, other things being equal, according to the agglutinative strength of the blood it is evident that methods of exact mensuration of this strength must take into con-

sideration the length of time required for the development of the reaction after the addition of the serum. Many writers have not paid much attention to this point. Stern has proposed that a limit of two hours be adopted as an arbitrary standard for the microscopic reaction, and Widal has accepted this proposal. With this unit, an agglutinative power fixed at 1 to 500 means that 1 part of serum added to 500 parts of the fluid containing the culture, agglutinates and paralyzes the bacilli within two hours, although a higher dilution may give a decided reaction in six or eight hours. The optimum effect is, according to Stern, not attained before the lapse of six or eight hours.

By varying the time limits, results obtained by lower dilutions may be roughly comparable with those by higher dilutions. Thus, for diagnostic purposes, a fifteen minute time limit for dilutions of 1 to 10 may be adopted and a two hour time limit for dilutions of 1 to 50 or higher, but it should be understood that in all doubtful cases quantitative determinations by varying the dilution should be employed.

Criteria of the reaction.—Some writers have proposed to make either the cessation of motility or the clumping the essential criterion of the reaction. Thus Stern selects the clumping and Kühnau the paralysis of motion. In my judgment both phenomena enter equally into the reaction and deserve equal consideration, so that a reaction is not to be considered complete and satisfactory unless the bacilli are both clumped and rendered immobile. Partial reactions in which one or the other characteristic is lacking may warrant suspicions and lead to further examination, but they should not be made the basis of positive diagnosis. For this reason the use of killed cultures, as suggested by Widal, while it may have a limited field of application, can not supplant the ordinary method. As already stated, the microscopic reaction is to be preferred to either of the macroscopic methods, although the latter afford striking

objects for demonstration. With low dilutions bactericidal and lysogenic phenomena are common, but they do not pertain to the agglutinative reaction itself.

Certain physical conditions influencing the reaction.—As already mentioned Salimbeni has shown that free exposure to the air favors the reaction and Widal has pointed out that partial evaporation at the edge of the cover-glass and contact of the specimen with slide and cover-glass are also favoring conditions. Hence, the conditions for the reaction are not exactly the same with the serum bouillon mixture in thin layer beneath the cover-glass on an ordinary slide, as in a thick layer, or in a sealed drop culture on a hollow slide, or in a column of fluid in a test-tube, or in a moist chamber. The presence of fibrinous masses, granules and material foreign to blood serum may perhaps explain in part the greater frequency of partial reactions with normal blood when the dried blood method is used than when the serum method is employed. The temperature of the incubator, by favoring evaporation and in other ways, accelerates the reaction. For exact quantitative work these various physical conditions need consideration and further investigation. Widal prefers the use of ordinary slides to that of hollow-ground slides, and does not advise keeping the specimen in the incubator. It is not to be supposed that the diagnostic use of the serum test generally hinges on such delicate points as these, but they are among the points to be considered in the explanation of certain irregularities in the results of the test, in comparing the results of different workers, and in mensuration of agglutinative power, especially with high dilutions.

Reactions with the colon bacillus.—Statements of different writers as to the occurrence of the agglutination of the colon bacillus with normal and typhoid sera are not harmonious. Widal and Courmont find that all human sera, whether normal or typhoid, have a slight agglutinating action on the colon bacillus in dilution of 1 to 10, whereas normal sera have only

exceptionally any such action on the typhoid bacillus in this dilution. Many observers have noted some agglutination of colon bacilli with typhoid serum, although the reaction is much less intense than with the typhoid bacillus. Vedel found, in a case with symptoms of typhoid fever but without the typhoid serum reaction, marked colon reaction, and he interpreted the case as one of colon infection simulating typhoid. He is not, however, inclined to attach much diagnostic importance to the colon reaction, as he found that it might be well marked both with normal and typhoid blood. Johnston and McTaggart found genuine colon reactions with typhoid blood to be rare, provided the typhoid reaction was well marked. In several cases, however, where the symptoms suggested typhoid but the typhoid serum reaction was absent, they found marked colon reaction. They are inclined, therefore, to attach diagnostic importance to the latter reaction. The colon cases were mild and of shorter duration than ordinary typhoid fever.

Kühnau makes use of the colon reaction to assist in the diagnosis of typhoid fever in doubtful cases. He finds that normal serum reacts in the same way with both colon and typhoid bacilli, whereas typhoid serum, even when of weak specific power, reacts much more intensely with the typhoid bacillus than with the colon bacillus. By availing himself of this unequal action of typhoid serum upon the two species of bacteria, he felt justified in making the diagnosis of typhoid fever when the agglutinative power of the serum did not exceed 1 to 20. Kühnau's suggestion is interesting, but further investigations are needed to determine its value.

We must also await further studies before Johnston and McTaggart's highly suggestive views as to the existence of colon infections simulating typhoid and capable of diagnosis by the serum reaction with the colon bacillus can be accepted.

As was first pointed out by me in 1890, the colon

bacillus is an extremely common secondary invader of the body in all sorts of conditions, particularly those with lesions of the intestine. It can very frequently be found in internal organs outside of the intestine in typhoid fever, if careful search is made. We have no satisfactory proof that it produces either symptoms or lesions in most of these cases, and one would expect more common and intense serum reactions with bacillus coli in typhoid fever, if the organism was engaged in pathogenic work. The writer has repeatedly taken occasion to protest against what seem to him unwarranted inferences as to the pathogenic significance of the mere detection of the colon bacillus in the internal organs at autopsies, although there can be no question that under certain conditions this bacillus may be pathogenic for man.

As the colon group of bacilli contains numerous races, some approaching the typhoid bacillus closely, it is to be expected that they will vary markedly in their sensitiveness to agglutination with different sera.

Durham found that typhoid immune serum in no instance produced any agglutinative reaction with ten different specimens of the bacillus coli obtained from various sources. Colon immune serum reacted on its own race of bacilli exactly like typhoid serum on typhoid bacilli, but it did not react with all races of colon bacilli, a graduated series of effects being observed with different specimens of these bacilli. Rodet, however, whose results are reported in much less detail than those of Durham, found a certain degree of reciprocal action between colon and typhoid immune sera and their respective bacteria.

Date of appearance and disappearance of the typhoid serum reaction. Absence of reaction.—The presence of the specific agglutinative reaction can usually be counted on by the end of the first or the beginning of the second week of typhoid fever. It may appear as early as the second day of the disease (Johnston and McTaggart, C. Fraenkel), but this is very exceptional. It may here be noted that the

determination of the exact day of a disease, often so gradual and insidious in its development as typhoid fever, must frequently be more or less arbitrary, and will vary according to the case and with different observers. Sometimes the first appearance of the reaction is delayed, exceptionally until the end of the second or into the third week, or even later. There are rare cases in which the reaction is missed during the first attack and makes its appearance in the relapse (Breuer, Thoinot, Biggs and Park, and others). It has even been missed until the first days of convalescence. Blumenthal relates an interesting case in which the reaction was absent during the fever, tests being made on the twelfth and twenty-first days with serum dilutions of 1 to 10, but it was found with dilutions of 1 to 100 two days after the beginning of apyrexia. Achard likewise once found the reaction only during convalescence. We have not at present a sufficient number of accurate data to furnish definite figures as to the frequency of these delayed reactions, but their occurrence undoubtedly constitutes a defect in the method of serum diagnosis of some importance. A negative result of the test does not exclude the diagnosis of typhoid fever. The probability against this diagnosis is the greater, the later the period of the fever in which the negative result is obtained and the oftener the examinations are repeated. As regards the interpretation of negative reactions, the serum test does not differ from other bacteriological diagnostic tests, that for the tubercle bacillus for instance.

There are authentic cases of typhoid fever in which repeated examinations of the blood during the course of the disease and its convalescence failed to reveal the specific agglutinative reaction, even with serum dilutions of 1 to 10. We can not at present say what percentage of the total number they make. Widal and Sicard found absence of the reaction in only one out of 163 cases of typhoid fever examined by them. In this negative case, in which the diagnosis was confirmed by cultivation of typhoid bacilli obtained by

hypodermic puncture of the spleen, the reaction was absent during the fever, the apyrexia, the relapse and the convalescence. Of 116 cases of typhoid fever examined by Courmont, the reaction appeared in all, being delayed after the eighth day in only five. Of 70 cases examined by Chantemesse, it was present in all. Of 129 cases examined by Johnston and McTaggart, if a few cases examined only late in convalescence, or at a very early stage without reëxamination, be excluded, the reaction was missed in only one. In many reports cases, believed to be typhoid, are recorded as giving negative reaction when only one examination was made, this being sometimes early in the disease. Such cases doubtless belong mainly to the group with delayed reaction. The importance of repeated examinations is illustrated by such observations as Stern's, in which the test was negative at the end of the second week and positive two days later; of Widal's, negative on the tenth, positive on the twenty-second day, and several others of similar purport.

The agglutinative power of the blood tends to increase during the progress of the fever, but there are exceptions, and in general the intensity of the reaction is subject to irregularities and oscillations, which may be notable from day to day. There may be marked sudden rise or fall of reactive power. While weak reactions are more common in mild cases, there is no definite correlation between premature or delayed development or the intensity of the reaction and the gravity of the disease. The persistence of high agglutinative power, for example 1 to 2,000, after subsidence of the fever, does not prevent relapses.

In the majority of cases the specific agglutinative power of the blood diminishes in the first weeks or months after cessation of the fever and disappears within a year. Exceptionally it may vanish as early as eight or ten days after the fever. Widal and Sicard noted its disappearance on the eighteenth and twenty-fourth days, Breuer on the seventeenth and twenty-

fifth days, E. Fraenkel on the twenty-eighth day after defervescence, etc. Disappearances at such early dates as these are, however, not the rule. According to Courmont's experience, the serum reaction disappears in children most frequently during the course of the first two months, and in adults toward the fifth and sixth months, although it is not uncommon for it to continue a year. The specific reaction may, however, persist for years, perhaps indefinitely. Of forty cases which had had typhoid fever at least a year before examination, Widal and Sicard found the agglutinative reaction, either marked or slight, in eleven: after one and one-half year, one case, reaction weak; after two years, one case, reaction weak; after three years, two cases, in one marked, in the other weak; after six years, one case, reaction 1 to 10; after seven years, one case, reaction marked; after eight years, one case, reaction 1 to 1,800; after nine years, three cases, one marked, one 1 to 40, one 1 to 30; after twenty-six years, one case, 1 to 30. Kühnau found after one year, two cases with reaction of 1 to 80; after two years, one case, reaction 1 to 60; after seven years, one case, reaction 1 to 60. All others of a series examined (total number not stated) showed negative reaction after one year. It was observed by Widal and Sicard that in contrast to the reactions during infection and for the first weeks after defervescence, those of long standing showed no notable fluctuations in intensity during the periods of examination, extending sometimes over several weeks.

The persistence of the specific reaction after typhoid fever is of importance from two points of view, retrospective diagnosis and interpretation of the diagnostic significance of the reaction during a febrile infection. It is sometimes of interest and practical importance to determine that an individual has previously had typhoid fever. Thus Courmont was able by the serum diagnosis to determine that a patient with multiple neuritis, supposed to follow an attack of dysentery, was in reality convalescent for a month and a half

from typhoid fever, and Achard recognized the real nature of an attack of osteomyelitis in a patient who had had typhoid fever a year before (cited from Widal and Sicard)

Many writers have called attention to the evident possibility of a mistake in diagnosis when the serum reaction is found in a person with a febrile infection, who has recovered from typhoid fever, although it does not appear that any serious difficulty has been encountered thus far from this source of error. It, however, indicates the importance of obtaining a careful history of the patient, not only as regards recognized typhoid fever, but as to attacks interpreted as dysentery, gastric fever, appendicitis, malaria, etc. An observation reported by Stern indicates that the specific typhoid reaction may be acquired even without manifest illness. He suggests that careful quantitative estimations of agglutinative power may restrict the possibility of error in diagnosis arising from long persistent reactions, as increase or diminution in the course of the fever or of the convalescence would speak for fresh infection. Even if the fullest possible allowance be made for this source of error, it applies to so small a number of cases that the value of the method is not seriously impaired.

Presence of the reaction in non-typhoid cases.— The blood of many hundred persons, either healthy or affected with diseases other than typhoid, has been tested for the specific typhoid reaction, and it can now be asserted that a serum reaction which an experienced observer using accurate methods would consider characteristic of typhoid fever, is to be found only most exceptionally in those who have not had typhoid infection.

Several observers, especially the Germans, find that by adherence to Widal's original directions mistakes may occur, but that these can be avoided by attention to quantitative determinations, especially dilution of the serum, time limits, and characters of the culture used for the test. The most remarkable observations

on this point come from Breslau and are reported by Stern and Kühnau. Stern examined the blood serum of seventy persons not suffering from typhoid fever and, according to their statements, never having had typhoid fever. In twenty of these the serum had an agglutinative strength of 1 to 10, in five a strength of 1 to 20, and in two a trace of reaction was obtained with dilutions of 1 to 30. In none of these cases did he find any reaction with dilutions of 1 to 40. Of more than fifty similar cases examined by Kühnau, in forty-one no reaction was obtained with dilutions higher than 1 to 5. In eight agglutination was observed with dilutions of 1 to 10, up to 1 to 20; in four with 1 to 30; in three with 1 to 35, up to 1 to 40; and in one even with 1 to 50.

In judging these results, apparently so divergent from those of Widal and nearly all others, it is to be noted that both Stern and Kühnau used the microscopic reaction, made two hours the time limit, even for the lowest dilutions, kept the specimens in the thermostat, and that Stern regarded the clumping and Kühnau the paralysis of motion as the criterion of the reaction, thus apparently recording as genuine what many others would consider partial or pseudo-reactions. It is clear that those who interpret only complete reactions occurring within fifteen to thirty minutes at room temperature as genuine reactions, would not be likely to obtain any such results as those reported by Stern and Kühnau. Nevertheless, it must be admitted that even partial and late reactions are unwelcome and disturbing, even if an observer thinks that his skill and experience will enable him to avoid mistakes from their occurrence. It may also well be, as suggested by C. Fraenkel, that Stern's and Kühnau's normal and non-typhoid cases include not a few who had recovered from unrecognized typhoid infection, and this supposition is the more probable in consequence of the prevalence of typhoid fever in Breslau. Stern himself calls attention to the liability of failure to recognize certain mild cases.

There have been a few cases reported in which the diagnosis of typhoid fever was made on the basis of the serum reaction, but which the authors, from subsequent developments, considered to be free from typhoid infection. It has been claimed, therefore, that positive serum reactions are not a sure sign of typhoid fever, although no one claims that the chances of error from this source are more than slight. The cases reported by Achard and Bensaude, Jez, Ferrand, du Mesnil and van Ordt have given rise to the most discussion. For what seem to me justifiable criticisms of these reports, I would refer especially to the papers of Widal and of Stern, and I will limit my remarks to some general statements concerning such alleged failures of the serum test.

In the first place, as the matter now stands, positive reactions obtainable only with dilutions lower than 1 to 50, possibly than 1 to 60, especially if the reaction is partial and late in appearance, are not certain diagnostic signs of typhoid fever. In most of the cases just referred to no exact quantitative estimations of the agglutinative strength of the serum were made, and hitherto it has not been shown that the reaction ever occurs with non-typhoid serum in dilutions exceeding 1 to 50, with observance of other quantitative points which I have already considered. With the limited number of observations, however, which we now possess, we can not, of course, say but that such cases will be found in the future.

In the second place, infection with the typhoid bacillus can not be positively excluded either on clinical grounds alone or by anatomical examinations at the postmortem table. Infections with the typhoid bacillus occur without any characteristic anatomical lesions. There may be entire absence of ulcers or other lesions of the intestine. We have recently had at the Johns Hopkins Hospital a case with positive serum reaction, from which Dr. Flexner cultivated, in large number, typical typhoid bacilli from the gall bladder although there was no previous history of typhoid

fever, and there were no intestinal lesions. In 1891 I called attention to the favorable conditions offered by the bile for the prolonged survival of the typhoid bacillus. In one case I was able to demonstrate large numbers of typhoid bacilli in the bile of a rabbit, 128 days after intravenous injection of 0.5 c.c. of a bouillon culture[4]. Pick has reported a case with marked positive serum reaction in which at the autopsy no typhoid intestinal lesions and no swelling of the spleen were found, but bacteriological examination showed the presence of typhoid bacilli, not however in the spleen. Guinon and Meunier's case is instructive. During life the symptoms were those of acute miliary tuberculosis and typhoid fever combined. Serum reaction was positive. At autopsy the lesions appeared to be only those of acute miliary tuberculosis, small ulcers in the intestine being typically tubercular in aspect. Typhoid bacilli, however, were cultivated from the spleen and other parts. As both the symptoms and the bacteriological examination indicated that the typhoid infection was in course of disappearance, the case, if examined at a somewhat later period, might readily, as Guinon and Meunier remark, be placed to the discredit of the positive value of the serum test. We are justified, in the light of such cases as these, in demanding that thorough bacteriological examinations be made before cases which have given during life the characteristic serum reaction, but which do not present at autopsy the anatomical lesions of typhoid fever, be recorded as free from infection with the typhoid bacillus.

In the third place, the difficulty of excluding a previous attack of typhoid fever, after which, as already stated, the specific serum reaction may persist for years, is to be borne in mind.

In conclusion, I would emphasize the following practical points:

1. Experience has demonstrated that the method

[4] Welch: Bulletin of the Johns Hopkins Hospital, August, 1891.

of serum diagnosis of typhoid fever is of great practical value.

2. The alteration of the blood on which this method is based, is a specific effect of infection or intoxication with the typhoid bacillus.

3. The microscopic serum test is to be preferred to the macroscopic methods.

4. Quantitative determinations, relating especially to the culture, the time limits, and the dilution of the serum, are of importance and, at least in doubtful cases, should not be neglected.

5. As the reaction may be delayed or occasionally absent, a negative result of the test does not exclude the diagnosis of typhoid fever. The later in the course of the disease the test is applied, and the oftener the examinations are repeated at intervals, the less is the probability of the existence of typhoid fever.

6. The persistence of the reaction, sometimes for years, after recovery from typhoid fever, is to be borne in mind in interpreting the reaction in febrile conditions. The appearance of the reaction and its increase during the period of observation speak for fresh typhoid infection.

7. The danger of mistakes from positive reactions in non-typhoid cases can be guarded against in nearly all cases.

8. Provision should be made, especially by the establishment and support of municipal or State laboratories, to render generally available to practitioners the serum method of diagnosis, as well as other bacteriologic procedures of similar practical value.

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